Supplemental Document

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Two-photon microendoscope for label-free imaging in stereotactic neurosurgery: supplement

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TWO-PHOTON MICROENDOSCOPE FOR LABEL-FREE IMAGING IN STEREOTACTIC NEUROSURGERY: SUPPLEMENTAL DOCUMENT

1. ZEMAX Design of GRIN Microendoscope

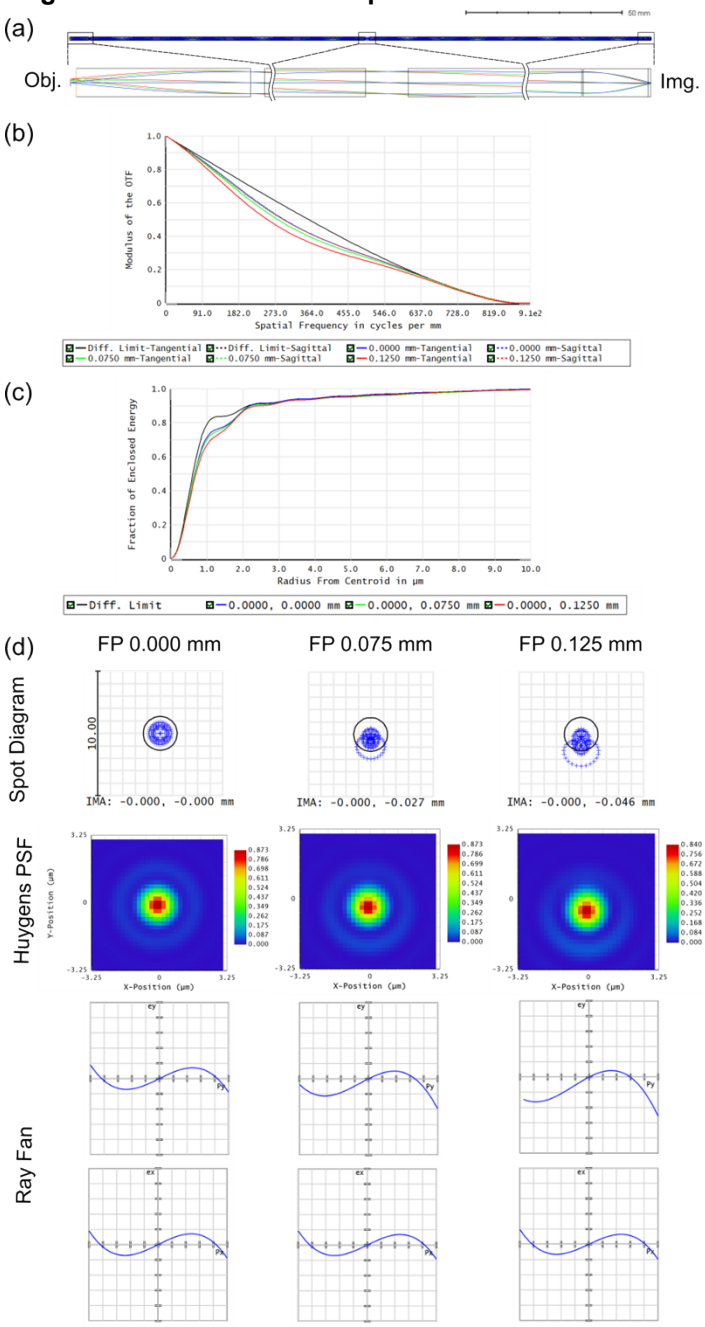

Fig. S1. (a) ZEMAX OpticStudio diagram of the excitation path through the GRIN Microendoscope at 900 nm. Three object field points (FP) were modeled: 1) blue (x = 0.000 mm, y = 0.000 mm), 2) green (x = 0.000 mm, y = 0.075 mm), and 3) red (x = 0.000 mm, y = 0.125 mm), (b) Fast Fourier Transfer Modulus of the Optical Transfer Function (OTF), (c) Encircled Energy, and (d) Spot diagram with airy circle, Huygens Point Spread Function (PSF), and Ray Fan aberration diagrams for the different object field positions.

2. Immunohistochemistry of Post-Mortem Human Brain Tissue

Many different metabolites and cofactors can generate endogenous fluorescence. We hypothesized that the primary source of the autofluorescence was intracellular granules of lipofuscin due to the broad emission spectra (Fig. 6c) and the resistance to photobleaching (data not shown). Lévesque and Parent previously found lipofuscin granules in the neuronal cells of the STN in normal post-mortem, human brain tissue [1]. Additional studies indicate that lipofuscin progressively accumulates in both neurons and glial cells in nearly all neuronal tissues [2]. We performed immunohistochemistry and staining to identify the cellular location of the presumed lipofuscin granules in fixed, human tissue samples from the STN. Microglia were labeled with ionized calcium binding adaptor molecule 1 (Iba1) and Nissl was applied to label granules in the cytoplasm of nerve cell bodies.

Imaging was performed using a Leica TCS SP5 II laser scanning confocal microscope using a 20X, 0.7 NA air objective (Fig. S2a) and a 63X, 1.40 NA oil immersion objective (Fig. S2b). Imaging of the granules was consistent with lipofuscin as the signal was present in multiple color channels (Blue, Green, and Red) that were colocalized. We concluded that most lipofuscin granules were located intracellularly in microglia or neurons (Fig. S2).

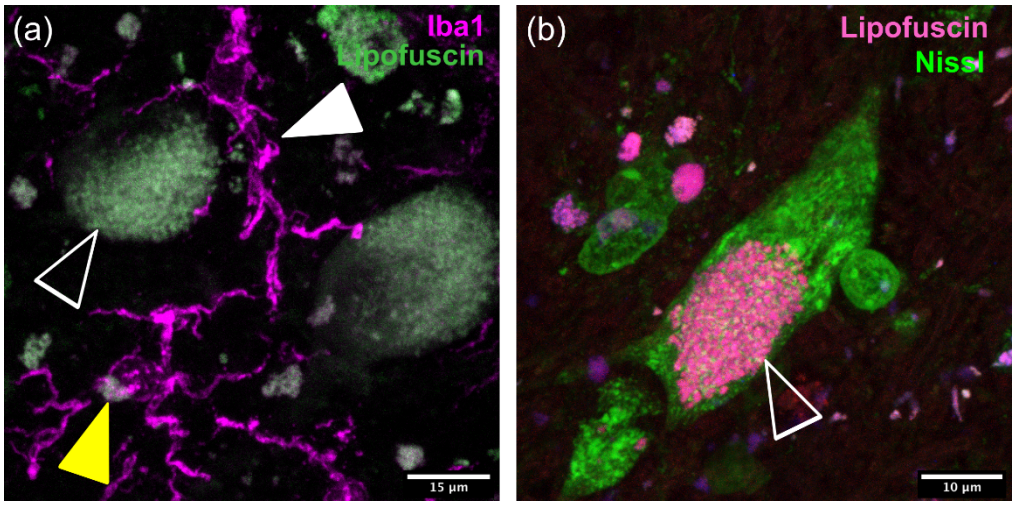

Fig. S2. Immunohistochemistry of human brain samples. (a) Image of microglia (Iba1, magenta) and lipofuscin (green/grey) in the human STN. Filled, white arrowhead indicates microglia contacting a cell containing a large lipofuscin cluster. Open, white arrowhead shows a cluster of lipofuscin granules in a cell. Yellow arrowhead indicates a smaller lipofuscin granule located in the cell body of a microglia. (b) Zoomed in view of Nissl and lipofuscin in the STN. Open, white arrowhead indicates large lipofuscin granule cluster located inside the Nissl^{+} region. Based on the size and staining pattern of the Nissl, this is likely a neuron.

Immunohistochemistry and Staining Protocol

Free floating sections of brain tissue were washed three times for 5 minutes in 1X Phosphate Buffered Saline (PBS) before being transferred to a beaker containing antigen retrieval solution (10 mM Sodium Citrate in 0.5% Tween). The beaker was placed into a water bath at approximately 93°C for 20 minutes. The temperature of the bath was monitored periodically and adjusted to keep the solution from boiling. After 20 minutes, the beaker was removed from the bath and placed at room temperature for 20 minutes. The tissue was washed three times in 1X PBS for 5 minutes and permeabilized for 30 minutes in 1% TritonX-100. After three additional washes in 1X PBS, the tissue was placed in blocking solution (5% Normal Donkey Serum (NDS) in 0.3% PBST) and incubated for 1 hour at room temperature. After blocking, tissue sections were placed in primary-antibody-containing blocking solution (Table S1) on an

orbital shaker at 4°C for 1 to 4 days depending on the thickness of the tissue section. After primary antibody incubation, sections were washed three times in 1X PBS for 5 minutes and incubated in secondary antibody (Table S1) for 1-2 hours on an orbital shaker at room temperature. After three final, 5-minute washes in 1X PBS, the tissue sections were mounted with VECTASHIELD Plus, and the coverslip was secured with nail polish.

Table S1: Antibody Information

Target	Primary Antibody	Concentration	Secondary Antibody	Concentration
Microglia	Goat anti-Iba1 (Cat. ab5076, Abcam)	1:400	Donkey anti-Goat (488 nm) (Jackson)	1:500
			Donkey anti-Goat (647 nm) (Jackson)	
	Rabbit anti-Iba1 (Cat. 019-19741, Wako)		Donkey anti-Rabbit-(594 nm) (Jackson)	
Neurons	Rabbit anti-MAP2 (Cat. ab5622, Millipore)	1:200	Donkey anti-Rabbit (594 nm) (Jackson)	1:500

A subset of samples was also labeled with FluoroNissl™ (cat. N21482, Invitrogen NeuroTrace™) to visualize cell bodies. Nissil sections were permeabilized and washed per the procedure above. FluoroNissl was diluted to a concentration of 1:25 in 1X PBS. The tissue slices were incubated in Nissl at room temperature for 1 hour, washed in 1X PBS for 2 hours, and mounted for imaging.

References

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